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Viral Immune Complexes in Systemic Lupus Erythematosus: C-type Viral Complex Deposition in Skin

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Punch biopsies were examined by indirect immunofluorescence for immune complex deposits containing C-type viral antigen. Antisera specific for immunoglobulins and HEL-12 virus mediated fluorescence at the dermal-epidermal junction and in vessel walls in 16 of 16 biopsies of involved skin from patients with systemic lupus erythematosus (SLE). Preimmune sera did not mediate fluorescence and gradient purified HEL-12 virus, simian sarcoma virus and baboon endogenous virus but not Rous sarcoma virus blocked the reaction of anti-HEL-12 virus serum with SLE tissue. Ten biopsies from uninvolved skin of the patients with SLE did not react with the antiviral serum, nor did tissue from 9 patients with discoid lupus erythematosus, psoriasis, bullous pemphigoid or normal skin. These data support the hypothesis that C-type viral immune complexes participate in the pathogenesis of SLE.

The etiology of systemic lupus erythematosus (SLE) is presently unknown. The hypothesis that C-type viruses participate in the pathogenesis of SLE [1,2] has recently received support from findings of C-type viral antigen containing immune complexes in lupus nephritis [3,4] and of widespread anti-C-type viral antibody in human sera ([5,6] Prochownik EV and Slamon D, personal communication).

We detected immune complexes in 31 of 32 cases of lupus nephritis using an antiserum raised to HEL-12 virus [4]. In contrast, only 4 of 65 control cases, including normal tissue and 35 cases of nonSLE immune complex nephridities reacted with the viral immune serum. The reaction was obliterated by prior absorption of antiserum with purified primate C-type viruses or extracts of virus infected cells but not extracts of control uninfected cells. Antibody eluted from SLE kidneys reacted with cells infected *in vitro* with HEL-12 virus [4]. Furthermore, antisera to 11 common viruses failed to detect antigens in SLE immune deposits.* These findings strongly suggest that C-type virus is expressed during the pathogenesis of renal disease in SLE. In addition, viral complexes were detected at multiple sites in 2 patients whose tissues were obtained post mortem, suggesting that C-type viral immune complexes may be widespread in SLE [7]. In an attempt to define how widespread C-type viral complex deposition is, we have now investigated the possible presence of viral complexes in skin biopsies from patients with SLE.

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Abbreviations:
BaEV: baboon endogenous virus
D-E: dermal-epidermal junction
DLE: discoid lupus erythematosus
PBS: phosphate buffered saline
SiSV: simian sarcoma virus
SLE: systemic lupus erythematosus
SR-RSV: Rous sarcoma virus

* Panem S, Ordóñez NG, Katz AI, Spargo BH, Kirsten WH, Laboratory Investigation, in press.

MATERIALS AND METHODS

Four millimeter punch biopsies of the skin were obtained from involved and uninvolved skin from 16 patients with diagnosed SLE. Involved tissue was taken from erythematosus macular or papular skin lesions of the face or arms. Uninvolved tissue was from unexposed skin of the buttocks or medial aspect of the arm. Tissue was also obtained from 3 patients with active discoid lupus erythematosus (DLE), 4 with bullous pemphigoid, 1 with psoriasis, and 1 normal individual. Biopsy tissue was immediately frozen in liquid nitrogen and stored at −20°C. Four to 6 μ thick sections were cut with a cryostat and were stained, unfixed for immunofluorescence [8].

Immunoglobulins and complement were detected by direct immunofluorescence utilizing fluorescein isothiocyanate conjugated antisera to human immunoglobulins (IgA, IgM and IgG) and human C3 [Hyland, Costa Mesa, California]. Frozen sections were prewashed in phosphate buffered saline (PBS) pH 7.2 for 10 min at room temperature. Fluorescein conjugated antisera were then incubated with sections for 30 min in a humid chamber. Each slide was then individually rinsed with PBS utilizing a squeeze bottle and washed twice for 10 min in a PBS bath using a magnetic stirrer. Coverslips were mounted in gelvatol.

Viral antigens were detected by indirect immunofluorescence with pre-immune rabbit serum or rabbit-anti-HEL-12 virus serum as the primary serum and goat-anti-rabbit-IgG-fluorescein isothiocyanate [Meloy Laboratories, Springfield, Va.] as the secondary serum. All sera (preimmune and immune sera, fluorescein conjugates) were absorbed twice with mouse liver powder prior to dilution [4]. The preparation of the anti-HEL-12 virus serum has been described [4]. The immune serum, but not pre-immune serum, reacts with virus infected cells but not uninfected cells and with immune deposits in lupus nephritis. The reaction of immune serum with lupus immune complexes cannot be removed by prior absorption of serum with human serum albumin, human immunoglobulins or fetal calf serum although it can be specifically blocked by HEL-12 viral proteins* [7]. Absorption of HEL-12 viral serum with human serum from 8 normal subjects did not block the reaction with SLE immune deposits. The technique for indirect immunofluorescence is parallel to that described above for direct fluorescence, with the primary serum being applied for 30 min, washed as described, the secondary serum applied for 30 min and washed as described before coverslipping.

Specificity of immunofluorescence reactions were tested by serum absorption. Increasing concentrations of gradient purified viruses (3–60 μg/100 μl anti-HEL-12 virus serum diluted 1:20) were incubated for 60 min at room temperature and overnight at +4°C. Antisera were then tested on replicate sections of an SLE skin biopsy as described above. The viruses used were simian sarcoma virus (SiSV) grown in 71API cells; the M7 strain of baboon endogenous virus (BaEV) grown in BILN cells; and the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV), purchased from Electronucleonics, Bethesda, Md. HEL-12 virus spontaneously released from HEL-12 cells was purified by 2 consecutive cycles of gradient centifugation as previously described [9].

All specimens for immunofluorescence were evaluated under code independently by 2 individuals. Positive samples were photographed utilizing Kodak high-speed daylight ektachrome film, ASA 200. Samples were judged positive, negative, or trace. The microscope used for evaluation was an AO-10 Microstar American Optical microscope with a high-tension mercury lamp as a light source, fitted with BG-12 and UG1 filters.

RESULTS

Thirty-seven skin punch biopsies were evaluated by immunofluorescence for deposition of immune complexes which reacted with antisera to HEL-12 virus (Table I). Sixteen of 16 biopsies taken from involved skin of SLE patients reacted with antisera to human immunogobulins. Several of these samples were also examined with antisera specific for the C3 complement component and were found to be positive (data not presented). In these biopsies, immunoglobulins and complement were detected at the dermal-epidermal (D-E) junction. Occasionally, fluorescence was also noted in walls of vessels. Each of the SLE specimens which showed immune complex deposition reacted with the antiserum to HEL-12 virus. The end-point dilution of SLE immune deposits at the D-E junction varied from 1:10 to 1:60. In contrast, no specimen reacted with the preimmune rabbit serum. The pattern of immunofluorescence mediated by the anti-HEL-12 virus serum was identical to that seen for immunoglobulins and complement (Fig 1*A* and *B*). As shown in Fig 1*B* and 2, granular fluorescence was found at the D-E junction and in some cases in vessel walls with anti-HEL-12 virus serum. In contrast to these findings, 10 samples taken from uninvolved skin of the same patients failed to react with the anti-HEL-12 virus serum although antiserum to human immunoglobulins reacted with all of these samples.

No reaction was observed between the HEL-12 virus serum and skin taken from a normal patient or with rhesus monkey esophagus. Samples of these tissues were included in all staining experiments as negative skin controls. Furthermore, prior absorption of anti-HEL-12 virus serum with minced rhesus monkey esophagus did not remove the ability of anti-HEL-12 virus to react with SLE immune deposits. A sample of SLE kidney tissue which contains immune deposits reactive with anti-HEL-12 virus serum was always included as a positive control for the

TABLE I. *Detection of HEL-12 viral antigen at the dermal-epidermal junction*[a]

Diagnosis	No. of cases	Immunofluorescence with antisera to	
		IgG + IgA + IgM	HEL-12 virus
SLE			
Involved	16	16	16
Uninvolved	10	10	0
DLE	3	3	0
Bullous Pemphigoid	4	4	0
Psoriasis	1	0	0
Normal skin	1	0	0
Rhesus monkey Esophagus	1	0	0

[a] Frozen sections were examined as described in the Materials and Methods by indirect immunofluorescence for HEL-12 virus antigen and by direct immunofluorescence for immunoglobulin deposition. Preimmune rabbit sera acted as a negative control for the antiviral serum. Specimens of normal human skin or rhesus monkey esophagus were always included as a negative tissue control.

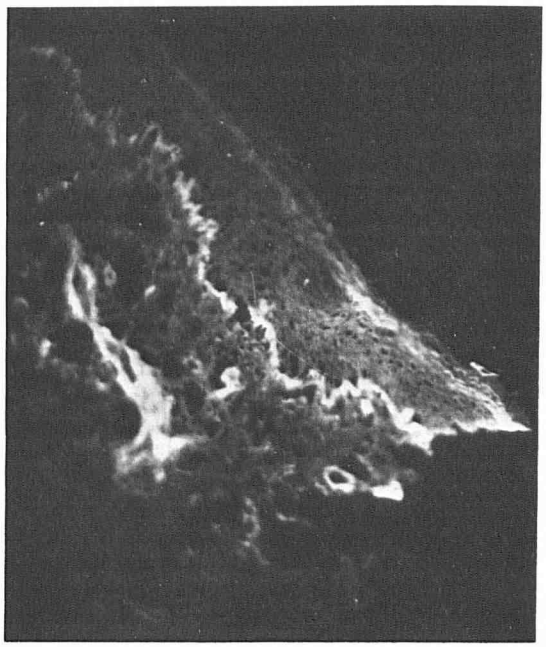

FIG 2. Indirect immunofluorescence demonstrating granular fluorescence at the dermal-epidermal junction and in the dermal capillaries with anti-HEL-12 serum (× 400).

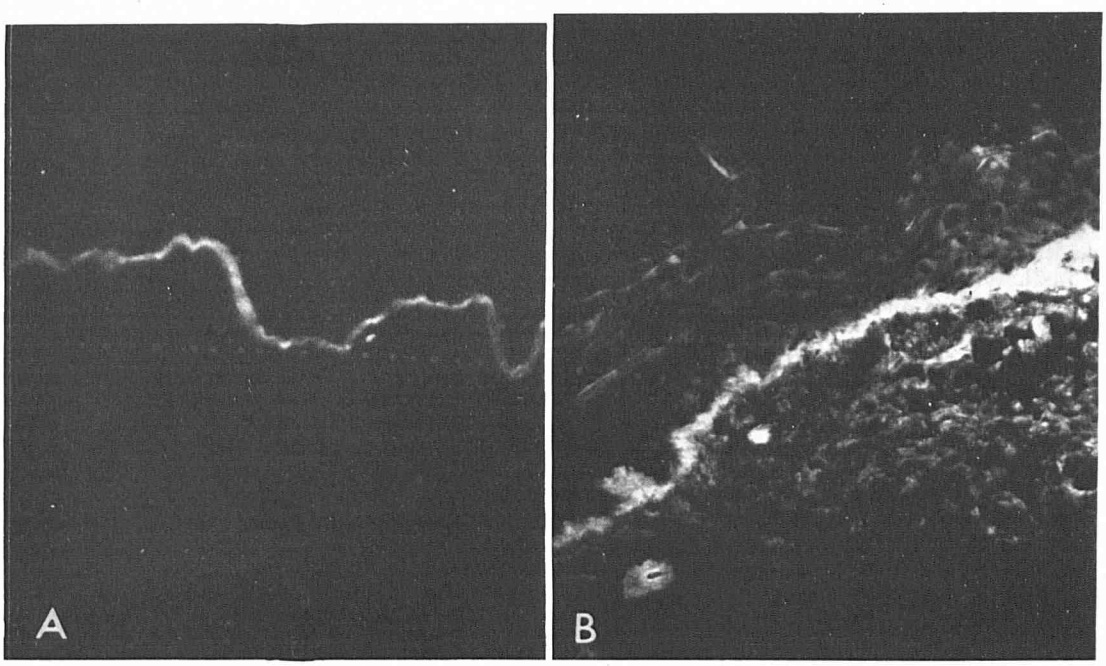

FIG 1. *A*, Direct immunofluorescence mediated by anti-human IgM-FITC at the dermal-epidermal junction of involved skin from an SLE patient. *B*, Indirect immunofluorescence demonstrating granular fluorescence at the dermal-epidermal junction in involved skin in the same SLE patient shown in *A* after reaction with anti-HEL-12 serum (× 650).

TABLE II. *Viral specificity of Immunofluorescence with anti-HEL-12 virus serum*[a]

Absorbent	Immunofluorescence at the dermal-epidermal junction
None	+[b]
Gradient purified virus:	
HEL-12 virus	
3 μg	+/−
15 μg	−
SiSV	
15 μg	+
60 μg	+/−
BaEV	
15 μg	+
60 μg	+/−
RSV	
15 μg	+
60 μg	+

[a] Anti-HEL-12 serum, final dilution 1:20, was absorbed for 60 min at room temperature and then overnight at +4°C. Absorbed sera were used in indirect immunofluorescence tests with sections of involved skin from SLE case 15S. Preimmune serum did not react with skin of this patient.

[b] Immunofluorescence was judged +, positive; −, negative, or +/−, trace, a distinct reaction which was too faint to photograph.

reactivity of the reagents. Under these conditions, the antiserum for HEL-12 virus failed to react with tissue of patients with bullous pemphigoid, psoriasis, and normal skin (Table I). Of special interest was the inability of the HEL-12 virus serum to react with 3 specimens from patients with DLE. The failure of the viral reagent to react with DLE lesions did not relate to the amount of immune complex deposition. DLE specimens showed equivalent or more immune complex deposition than SLE specimens as judged from the relative intensity of immunofluorescence mediated by polyvalent antisera to human immunoglobulins.

The specificity of the reaction between SLE immune deposits and anti-HEL-12 virus serum was tested by serum absorption studies (Table II). Antiviral serum was preabsorbed with increasing concentrations of HEL-12 virus; 2 primate C-type viruses which are antigenically related to HEL-12 virus, SiSV and BaEV; and the antigenically unrelated SR-RSV. Three micrograms of purified HEL-12 virus reduced the reaction and 15 μg obliterated the fluorescence mediated by a 1:20 dilution of anti-HEL-12 virus serum. In contrast, 60 μg of either BaEV or SiSV was needed to reduce the reaction. Sixty micrograms of SR-RSV did not affect immunofluorescence.

DISCUSSION

We have previously shown that immune complexes containing C-type viral antigen and antibody specifically correlate with lupus nephritis [4,7]. We have now demonstrated that viral antigen is deposited in association with immunoglobulin in SLE skin lesions. Twelve of the 16 SLE patients have had renal biopsies in which HEL-12 virus related antigen was detected. These findings, along with the detection of viral antigen and immunoglobulins along vessel walls and in heart, lung and conjunctiva of SLE patients sampled post mortem [7] support the hypothesis that C-type viral immune complexes are present in the circulation of patients with SLE and are deposited throughout the body.

We did not detect reactivity of the antiviral serum with the nucleus or cytoplasm of cells in either the dermis or epidermis, suggesting that the skin is not the site of viral antigen generation. This conclusion is also supported by the inability of the antiserum to react with tissue taken from uninvolved skin of SLE patients.

The specificity of the reaction of SLE immune deposits in skin and anti-HEL-12 viral serum was demonstrated by blocking the reaction by prior absorption utilizing HEL-12 virus and the antigenically related SiSV and BaEV but not the unrelated SR-RSV. Previously, we have failed to absorb the reaction of viral serum and SLE immune complexes utilizing human serum albumin, fetal calf serum, human immunoglobulins and extracts of uninfected human, rabbit and canine cells although purified HEL-12 virus and extracts of HEL-12 virus infected cells blocked the reaction. These experiences indicate that the antigen(s) reacting in SLE immune complexes are viral antigen(s). It is, however, important to note that C-type viral complexes are only one of many immune complexes which circulate and are deposited in SLE [10–12]. This observation is supported by our inability to find viral complexes in uninvolved skin although immunoglobulins and complement were detected in uninvolved skin of patients with SLE [12].

Previously, immune complexes in patients with over 16 different non-SLE nephritides failed to react with anti-HEL-12 virus serum* [4]. It was therefore not surprising that biopsies of patients with pemphigoid and psoriasis did not react. However, it was of interest that biopsies of 3 patients with DLE did not react. If this finding is borne out in a larger patient population it will be of importance to determine if C-type viral complexes are absent in these patients. For these reasons, studies on the composition of circulating complexes in SLE and DLE are now being pursued.

We originally examined tissues of patients with SLE for C-type viral immune complexes because C-type virus is known to participate in several syndromes studied in animals as models for SLE [1,2]. Our current data indicates that C-type viral immune complexes are present at sites of tissue injury in all patients with SLE that we have examined to date. However, in view of the increasing evidence for widespread C-type viral antigen and antibody expression in the normal human population, it is important to emphasize that the presence of C-type viral immune molecules correlates with more than SLE. More work is required before it can be determined that C-type viral expression is an obligatory component of SLE in man.

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